ELECTRON MICROSCOPE ANALYSIS OF THE DIMENSIONS OF MITOCHONDRIA DURING FLUCTUATIONS OF THE ION FLOWS

E. L. Kholmukhamedov and G. B. Khomutov

Vestnik Moskovskogo Universiteta. Fizika,
Vol. 34, No. 6, pp. 106-109, 1979

UDC 778.317:576.311,347

We present a method of calculating the true size of mitochondria according to the area of the sections obtained in electron photomicrography and give the results of such a calculation for mitochondria in a fluctuating state. The results obtained for mitochondria in different phases of the fluctuational cycle shows that the mean radii of mitochondria in the shrunken and swollen states differ by a factor of 1.8.

The attention of researchers in the field of bioenergetics at the present time is focused on the elucidation of the molecular mechanism underlying the occurrence of the fluctional functioning of mitochondria in vitro [1-4]. Analysis of the currently available results shows that one of the necessary conditions for the fluctuating state to occur is that there be motion of mono- and bivalent cations through the membrane of the mitochondria [3,4]. Because the inner membrane of mitochondria is osiotically active, one expects that the cyclical accumulation and discharge of K^+ and Ca^{2+} ions will lead to the increase and decrease of the volume of the mitochondrial matrix. The corresponding changes in the concentrations of the cations and of oxidation substrates localized in the mitochondrial matrix can turn out to have a substantial effect on the regularization of the state of the mitochondria and the generation of fluctuations. It is plain that a quantitative estimate of these changes cannot be done without a precise determination of the volume of the mitochondria and the extent of its variation during fluctuations.

In this paper we present a method of calculating the true size of mitochondria according to the area of sections obtained in electron photomicrography and give the results of such a calculation for mitochondria in the fluctuating state. The mitochondria were removed and fixed with glutaric aldehyde [5] at various points in their fluctuational cycle (see Fig. 1). The conditions of incubation and the methods of determining the concentration of ions were the same as in Ref. [4]: 20 mM saccharose, 1 mM KCl, 5 mM succinic acid, 12 mM triss-buffer, pH 7.5. The protein valinomycin was added to the mitochondria in the amount 5 ng/mg mitochondria.

Method of calculating the distribution of the true sizes of the mitochondria. The only method of directly observing the shape of mitochondria is that of electron microscopy. However, direct measurements of the cross-sectional area of the mitochondria in the photographs do not enable one to make any definite conclusions about the sizes of the mitochondria, since the areas of the cross sections depend on both the heterogeneity of the mitochondrial population and on the height of the cross section of the given mitochondrion (Fig. 2,*a*). The fluctuations of the ion flows occur in a hypotonic medium, and therefore the mitochondria swell and are spherical in shape. This is evidenced by th fact that all the cross sections of

the mitochondria are circular. On the basis of a large number of electron photomicrographs of mitochondrial sections, the experimental distribution of the probability density of cross sections as a function of their radius $P_{exp}(r)$ is constructed in the form of a histogram.

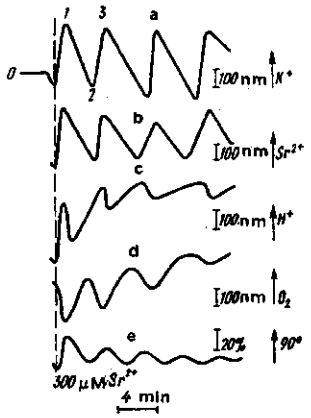

Fig. 1

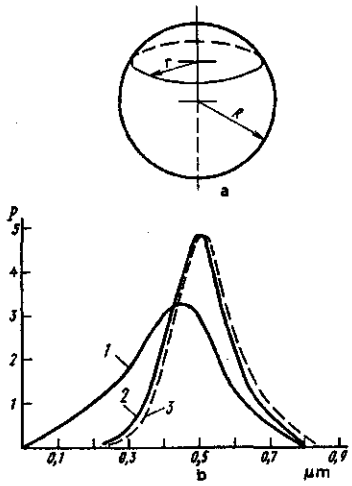

Fig. 2

Fig. 1. Fluctuations of the concentrations of ions (a, b, c, d) triggered by Sr^{2+} ions; curve e shows the change in the light-scattering of the mitochondrial suspension at an angle of 90°.

Fig. 2. Diagram of a section of a mitochondrion (a) and the size distribution of the mitochondria (b).

If it is assumed that the mitochondria are sectioned randomly in the process of preparing the samples for electron photomicroscopy, the probability density for obtaining a cross section of radius r for a mitochondrion of radius R (Fig. 2,a) is

$$g_R(r)\frac{r}{R\sqrt{R^2-r^2}}.$$

The probability $dF_{r,R}$ of obtaining a cross section of radius r if there are mitochondria of different sizes and the sectioned mitochondrion is of radius R has the form

$$dF_{r,R} = P_x(R)\,dR g_R(r)\,dr,$$

where $P_x(R)dR$ is the probability that the sectioned mitochondrion is of radius R.

A cross section of radius r can be obtained if any mitochondrion of radius $R \geq r$ is sectioned, and therefore, the total probability of obtaining a cross section of radius r, which can be determined on the basis of the electron photomicrographs, is equal to

$$dr\int_r^{R_{max}} P_x(R)\,g_R(r)\,dR = P_{exp}(r)\,dr,$$

where R_{max} is the radius of the largest sectioned mitochondrion (Fig. 2,b).

Thus, we obtain an equation connecting the distribution of the radii of the mitochondrial sections $P_{exp}(r)$ (curve 1), which is obtained from experiment, with the distribution of the radii of the sectioned mitochondria $P_x(R)$ (curve 3):

$$P_{exp}(r) = \int_r^{R_{max}} \frac{r}{R\sqrt{R^2 - r^2}} P_x(R)\, dR. \qquad (1)$$

The probability that a mitochondrion of radius R is sectioned is proportional to the linear dimension of the mitochondrion:

$$P_x(R) = kRP_0(R),$$

where $P_0(R)$ (curve 2) is the true distribution of mitochondrial radii in the volume of the sample; k is a constant of proportionality. The value of k can be obtained by integrating both sides over R;

$$\int_{R_{min}}^{R_{max}} \frac{1}{kR} P_x(R)\, dR = \int_{R_{min}}^{R_{max}} P_0(R)\, dR = 1,\ k = \frac{1}{\bar{R}},$$

where R_{max} and R_{min} are the maximum and minimum radii of the mitochondria in our electron photomicrographs, and $\bar{R}$ is the average radius of a mitochondrion in the distribution $P_x(R)$.

Finally, for calculating the true distribution of mitochondrial radii we obtain the expression

$$P_0(R) = \frac{\bar{R}}{R} P_x(R),$$

where the distribution $P_x(R)$ is found from Equation (1) on the basis of the experimentally obtained distribution of mitochondrial cross sections $P_{exp}(r)$ over radii r.

Results. To construct the distribution $P_{exp}(r)$ at each point (see Fig. 1) we took 500 mitochondrial cross sections. Equation (1) was solved numerically on a MIR-2 computer, and according to the values obtained for $P_x(R)$ the true distribution of mitochondrial radii $P_0(R)$ was constructed.

The data given in Fig. 2,b refer to point 0 on Fig. 1. Analogous results were also obtained for the other points (1, 2, and 3) indicated on the curves of Fig. 1.

Thus, we have resolved the question of the variations in the volume of mitochondria during fluctuations. Comparison of the results obtained by processing the areas of the mitochondrial sections in different phases of the fluctuational cycle shows that the average radii of the mitochondria in the shrunken and swollen states differ by a factor of 1.8, and the volumes by more than a factor of 5. These data show that in analyzing molecular mechanisms for the occurrence of the fluctuating states in mitochondria it is necessary to take into account the change in the volume of the mitochondria due to the accumulation and discharge of the ions taking part in the fluctuations.

In conclusion, the authors wish to thank G. A. Semenova for help in the preparation of samples for the electron microscope.

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27 June 1978

Department of Biophysics